

JEM 316

INTERNATIONAL STUDY ON *ARTEMIA*. XXXII. COMBINED EFFECTS OF TEMPERATURE AND SALINITY ON THE SURVIVAL OF *ARTEMIA* OF VARIOUS GEOGRAPHICAL ORIGIN¹

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Abstract: The brine shrimp inhabits geographically isolated biotopes with specific biotic and abiotic conditions. This has resulted in various geographical strains between which marked genetical, biological and chemical differentiation exists. The response of 13 different *Artemia* strains to the combined effect of temperature and salinity has been studied. Experimental temperatures tested ranged from 18 to 34 °C and salinities from 5 to 120‰.

Except for Chaplin Lake (Canada) *Artemia*, all strains showed high survival over a wide range of salinities (35–110‰). For all strains the common temperature optimum was between 20 and 25 °C. Interaction between temperature and salinity was negligible or very limited. Substantial differences in tolerance were recorded in particular at the lower end of the range of experimental salinities and at the upper end of the range of temperatures. Resistance to high temperature seems to be related to the genetic classification of the *Artemia* strains in different sibling species. Differences, however, also exist among strains from the same sibling species. Genetic adaptation to high temperature seems to take place in *Artemia*.

The data obtained provide a first guideline for strain selection for specific aquacultural purposes.

INTRODUCTION

The brine shrimp *Artemia* inhabits geographically isolated habitats distributed over the five continents in tropical and subtropical regions, as well as in boreal areas (Persoone & Sorgeloos, 1980). Substantial differences in water temperature and salinity have been recorded for different *Artemia* biotopes (Bowen *et al.*, 1978; Persoone & Sorgeloos, 1980). As a consequence, it can be expected that geographic isolation of brine shrimp populations in specific biotopes with different temperature and salinity conditions has led to populations with different tolerance ranges for these abiotic conditions. The differences in genetic constitution that have been recorded among *Artemia* strains

¹ International interdisciplinary study on *Artemia* strains coordinated by the *Artemia* Reference Center.

(Bowen *et al.*, 1978; Abreu-Grobois & Beardmore, 1980, 1982) further enhance the probability for physiological variations among strains.

Although *Artemia* is generally considered to be a euryhaline summer species (Carpelan, 1957; Ivleva, 1969; Bayly, 1972; Stephens & Gillespie, 1972), culture tests with five *Artemia* strains showed important differences in temperature and salinity requirements (Sorgeloos *et al.*, 1976; Claus *et al.*, 1977). A few ecological studies also indicate differences in temperature and salinity tolerance among *Artemia* strains (Anderson, 1958; Al-Uthman, 1971; Kristensen & Hulscher-Emeis, 1972). These ecological observations are not, however, sufficient to determine the requirements and tolerance of *Artemia*; that is the ecological data provided do not reflect the yearly, seasonal, and diurnal variation in temperature and salinity. Moreover, the occurrence of *Artemia* in nature is restricted to salinity levels high enough to restrict the presence of its predators.

The purpose of this study was to develop a standardized laboratory test procedure that allows simultaneous determination of preferences and tolerance ranges towards temperature and salinity for different *Artemia* strains. Such a study should lead to a better characterization of the different *Artemia* strains and provide guidelines for strain selection for specific aquacultural applications, i.e. inoculation in tropical regions, culture in media with specific temperature and salinity conditions.

MATERIALS AND METHODS

Thirteen geographical strains of *Artemia* have been studied. Details on their exact origin and some of their characteristics are provided in Table I. The cysts were always

TABLE I
List of *Artemia* strains studied: B, bisexual; P, parthenogenetic.

Source of cysts	Year of harvest (batch number)	Mode of reproduction ¹	Ploidy ²
San Francisco Bay (CA, USA)	1976 (288-2596)	B	2 n
Macau (Brazil)	1978 (871172)	B	2 n
Barotac Nuevo (Philippines)	1978	B	2 n
Great Salt Lake (UT, USA)	1977	B	2 n
Chaplin Lake (Canada)	1979	B	2 n
Buenos Aires (Argentina)	1977	B	2 n + 1
Larnaca (Cyprus)	1980	B	2 n
Barbanera (Spain)	1978	B	2 n
Shark Bay (Australia)	1977 (114)	P	2 n
Tuticorin (India)	1978	P	3 n
Lavalduc (France)	1979	P	2 n + 4 n
Tientsin (People's Rep. of China)	1978	P	2 n + 4 n
Margherita di Savoia (Italy)	1977	P	2 n + 4 n

¹ From Barigozzi & Tosi (1959), Clark & Bowen (1976), Bowen *et al.* (1978), Abreu-Grobois & Beardmore (1980, 1982).

² From Abreu-Grobois & Beardmore (1982), Barigozzi (pers. comm.).

incubated under optimal hatching conditions (25 °C; 35‰ S; 1000 lux; Sorgeloos, 1980).

Since tolerance to temperature and salinity is interrelated for several aquatic invertebrates (Kinne, 1970) a factorial design was used involving 25 combinations of five temperatures (18, 22, 26, 30, and 34 °C) and five salinities (5, 15, 35, 70, and 120‰). The media were prepared by use of the salt mixture of Dietrich & Kalle (1963 – in Kinne, 1970). To increase the buffer capacity, however, the quantity of NaHCO₃ was doubled. pH levels never dropped below 7.9 preventing any negative effects on survival and larval development as reported to occur at low pH (Provasoli & D'Agostino, 1969).

The tests were carried out in cylindro-conical tubes each containing 50 nauplii in 250 ml water. The tubes were aerated continuously from the bottom; they were covered with a perforated Petri dish to minimize evaporation. Temperatures were kept constant within ± 0.2 °C and salinities within ± 1 ‰. An acclimation period of 1 to 2 h seemed sufficient to prevent mortality from temperature shock. Since freshly hatched *Artemia* nauplii are very resistant to sudden salinity changes (D'Agostino & Provasoli, 1968; Sorgeloos, 1979) instar I nauplii were transferred directly to the different salinities. Each combination was tested in three replicates. For some strains, however, two extra replicates were added.

Larvae were fed *Dunaliella tertiolecta* Butch. cells each day throughout the 9-day experimental period. Since the metabolic needs of invertebrates may change as a function of temperature (Kinne, 1970) and salinity (Hernandorena, 1974), the optimal feeding regime at the experimental temperatures and salinities was determined in a series of preliminary experiments (Vanhaecke, 1983). No differences in food requirements could be detected as a function of salinity; the optimal feeding schedules for the different temperatures tested are given in Table II.

An experimental period of 9 days was deemed appropriate for the determination of the ranges of temperature and salinity tolerance, as the larvae may reach the preadult stage under the prevailing experimental conditions after 9 days. In addition, it appeared from the preliminary tests that further mortality was negligible beyond that period.

TABLE II

Feeding schedule (in 10^6 *Dunaliella* cells per day) for different test-temperatures (after Vanhaecke, 1983): no differences in food requirements could be detected in relation to salinity and this schedule was applied to all salinities tested.

	Temperature (°C)				
	18	22	26	30	34
Day 1	3.6	6.0	8.4	10.8	10.8
Days 2, 3, 4	7.2	12.0	16.8	21.6	21.6
Days 5, 6	10.8	18.0	25.2	32.4	32.4
Days 7, 8	14.4	24.0	33.6	43.2	43.2
Day 9	18.0	30.0	42.0	54.0	54.0

The survival results obtained after 9 days culturing were normalized through arcsin transformation $\sqrt{\%}$ (Zar, 1974). Statistical procedures included response surface analysis (Alderdice, 1972). Data were subjected to an analysis of variance from which the significance of the effect of temperature, salinity and the interaction between these two independent variables could be determined. The analysis of variance provided the basis for a regression equation describing the effects. The computer program "BOX 2", converted from an earlier version of the program of Lindsey & Sandnes (1972) for execution on a UNIVAC 1180/80 computer, was used for the analysis. Using likelihood inference, this procedure calculates the best fitting linear and non-linear mathematical equations to account for the variance. In the present study we used the non-linear model which provided a superior fit with higher coefficients of determinations (R^2) (Siddall, 1979). In view of high residual variances, calculated for some strains tested, five instead of three replicates were run for these *Artemia* strains. The program of Lindsey & Sandnes calculates the values of the independent variables at which maximum response (% survival) occurs. Substitution of the independent variables in the equation results in predicted response values that generate three-dimensional graphs of the resulting response surfaces. Contour plots are drawn for selected values of the response, i.e. 1, 10, 50, 70, and 90% survival.

According to Persoone *et al.* (1980) the binomen *Artemia salina* is no longer valid. Indeed, crossing experiments with different *Artemia* populations revealed reproductive isolation of several groups of populations and led to the recognition of sibling species to which different names have been given according to the International Conventions of Taxonomical Nomenclature (Bowen *et al.*, 1978; Bowen & Sterling, 1978). In this paper the *Artemia* populations have been grouped according to the species name designation of Bowen & Sterling (1978). Evidence of the exact sibling species of the strains studied was provided by Bowen *et al.* (1978) and Abreu-Grobois & Beardmore (1980, 1982).

RESULTS

ARTEMIA FRANCISCANA

San Francisco Bay (Fig. 1A), *Macau* (Fig. 1B), and *Barotac Nuevo* (Fig. 1C)

The *Artemia* strains from Macau and Barotac Nuevo originate from San Francisco Bay *Artemia* inoculated in the two former areas in 1977 and 1978, respectively (Sorgeloos *et al.*, 1979). From the analyses of variance for the three strains tested, both salinity ($P < 0.001$) and temperature ($P < 0.001$) significantly affect survival. The configuration of the contour plots, however, indicates that the salinity has little effect on survival except at the lower end of the test range of salinities.

The calculated temperature and salinity centres for maximal survival are, respectively, 20.6 °C at 62‰ for San Francisco Bay, 21.5 °C at 75‰ for Macau and 21.6 °C at 41‰ for Barotac Nuevo. It should be noted, however, that there is little change in response over a relatively broad range of salinities at the centre.

The differences in salinity tolerance are very limited. At least 90% survival is attained for both San Francisco Bay and Barotac Nuevo larvae within the range 15–120‰; for the Macau larvae good survival is recorded between 25 and 120‰. For the latter strain a small but significant ($P = 0.025$) low–low high–high interaction between temperature and salinity is observed, i.e. higher temperatures are tolerated better at the upper end of the salinity range.

Although the overall effect of temperature is similar for the three strains, subtle differences can be noted in particular at the upper end of the temperature scale. For the San Francisco Bay *Artemia* 50% mortality occurs at 29–30 °C whereas this is situated at 30–31 °C for Macau and at 32 °C for Barotac Nuevo *Artemia*. More than 90% survival occurs over a wide range of temperatures; 18 to 26–28 °C for Macau and Barotac Nuevo and 18 to 25 °C for San Francisco Bay. The data obtained for the latter strain match the results of Dobbeleir (1977). On the other hand, Sorgeloos *et al.* (1976), and Claus *et al.* (1977) noted only an increased mortality for San Francisco Bay larvae at temperatures exceeding 30 °C. This difference is probably related to the shorter duration of their culture experiments (3 and 4 days compared with 9 days).

Great Salt Lake (Fig. 1D) and Chaplin Lake (Fig. 1E)

The response surfaces for these strains of *Artemia franciscana* are very different from those of the previous strains of San Francisco Bay origin.

The survival of Great Salt Lake larvae is clearly influenced by salinity ($P < 0.001$). Between 15 and 120‰, the contour plots, however, are parallel with the salinity axis, pointing to an independence of survival with respect to salinity within this range. In accordance with the experimental data of Von Hentig (1971) Great Salt Lake larvae die below 15‰.

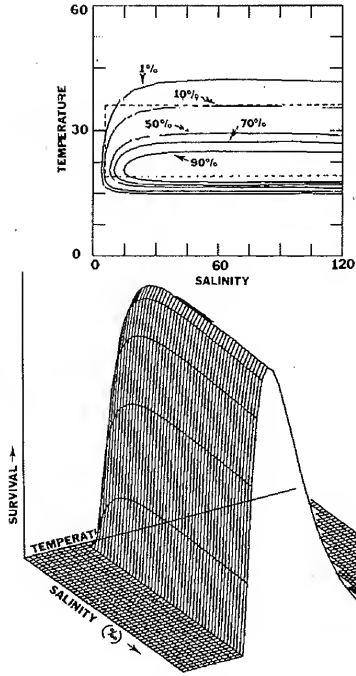
This strain is very tolerant to high temperatures. The contour plot corresponding to 70% survival extends close to 35 °C over a wide range of salinities. Survival exceeding 90% is predicted for a range of salinities from 30 to 120‰ at temperatures varying from 18 to almost 30 °C.

Contrary to Sorgeloos *et al.* (1976), who recorded a clear antagonistic interaction between temperature and salinity for Great Salt Lake nauplii, only a minimal interaction could be noted ($P = 0.04$); i.e. lower temperatures seem to favour slightly tolerance of low salinities.

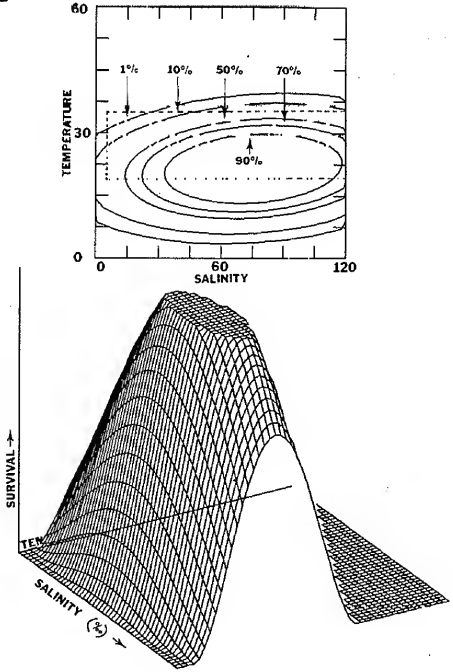
The response surface for Chaplin Lake *Artemia* substantially differs from that of all other *Artemia franciscana* strains studied. It is clear that temperatures < 18 °C have to be taken into account for the determination of the optimum temperature. Furthermore, the graphs illustrate that the salinity resistance of this strain is very limited. At temperatures varying between 18 and 22 °C, the optimal salinity range is between 45 and 75‰. None the less Chaplin Lake *Artemia* should not be considered as stenothermal. Within the optimal salinity interval 50% survival can be recorded at temperatures up to 30 °C. This is similar to the value obtained for the San Francisco Bay strain.

The analysis of variance indicates a significant temperature salinity interaction for

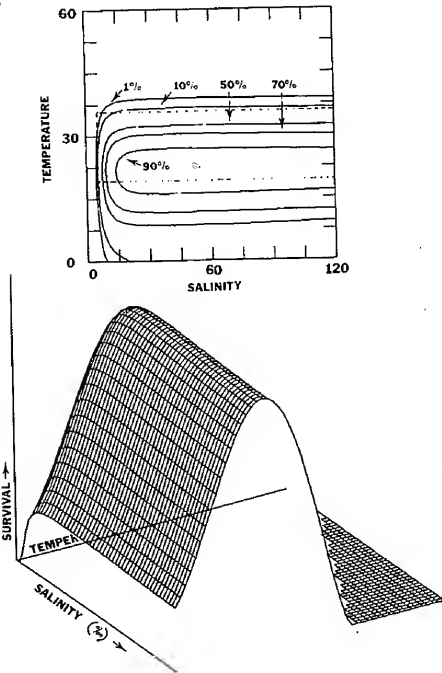
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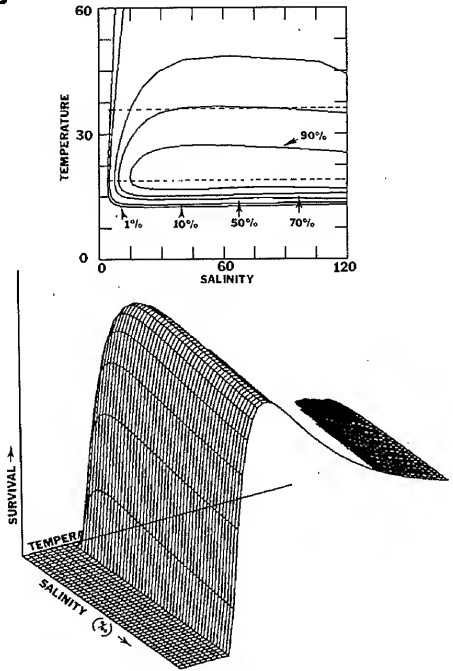
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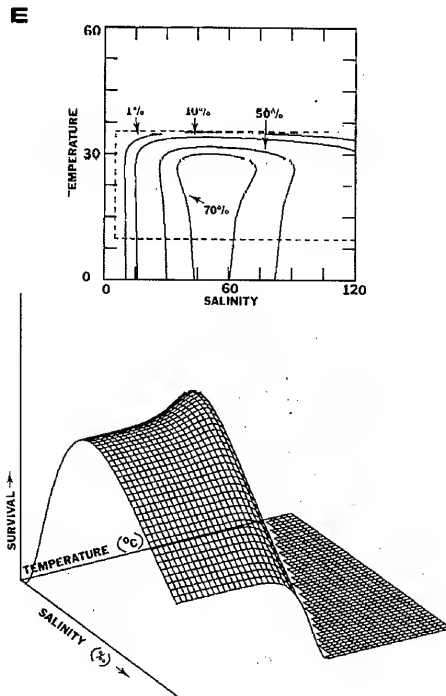


Fig. 1. Two-dimensional (upper) and three-dimensional (lower) graphical representation of response surfaces for survival of five *Artemia franciscana* strains as a function of temperature ($^{\circ}\text{C}$) and salinity (‰): isopleths of response (1, 10, 50, 70, 90% survival); A, San Francisco Bay; B, Macau; C, Barotac Nuevo; D, Great Salt Lake; E, Chaplin Lake.

Chaplin Lake nauplii ($P < 0.001$). An increased survival potential is associated with higher temperatures at the upper salinity range.

ARTEMIA PERSIMILIS: BUENOS AIRES (Fig. 2)

The *Artemia* strain from Buenos Aires is characterized by a high resistance to low salinities. Even at $5\text{‰} > 50\%$ of the larvae survive at both 18 and 22°C . Nevertheless within the range tested the effect of salinity is significant ($P < 0.001$). The calculated optimum salinity of 77‰ , however, has limited practical meaning.

The effect of temperature is significant ($P < 0.001$). This limited temperature resistance is illustrated by the small distance between the contour plots, corresponding to 90, 70, 50, and 10% survival. The 50% survival contour is at 29°C . At 34°C mortality is nearly 100%. The calculated temperature optimum is 22.5°C , but over 90% survival can be obtained between 19 and 26°C at salinities ranging from 60 to 90‰ . At lower salinities the optimal temperature range narrows. None the less, there is no significant interaction between the two independent variables ($P = 0.35$).

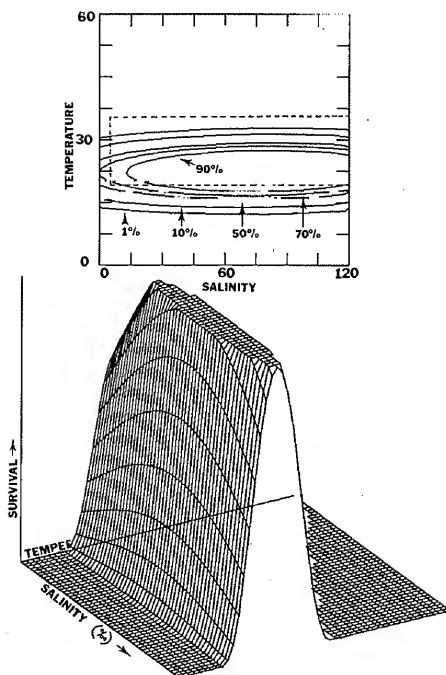


Fig. 2. Two-dimensional (upper) and three-dimensional (lower) graphical representation of response surfaces for survival of the *Artemia persimilis* strain from Buenos Aires as a function of temperature (°C) and salinity (‰): isopleths of response (1, 10, 50, 70, 90% survival).

ARTEMIA TUNISIANA: LARNACA (Fig. 3A) AND BARBANERA (Fig. 3B)

The configuration of the contour plots for the Larnaca larvae reveals a high salinity tolerance. From a salinity of 15‰ and above, very good survival is recorded. A similar observation can be made for the larvae from Barbanera, with this restriction, however, that they have a slightly smaller salinity range. Both strains have a low tolerance for high temperatures. Although the 50% survival contour goes as high as 27 and 28 °C all larvae die at 34 °C. Tolerance at lower temperatures seems to be relatively high.

For Larnaca *Artemia* the maximal survival response is at 21.7 °C and 42‰. Mortalities below 10%, however, were recorded in the temperature range between 18 and 25 °C at salinities between 15 and 120‰. Barbanera *Artemia* show maximal survival at 22.3 °C and 38‰. At 35‰ the temperature limits are 18 and 25 °C. No significant interactions between temperature and salinity could be detected.

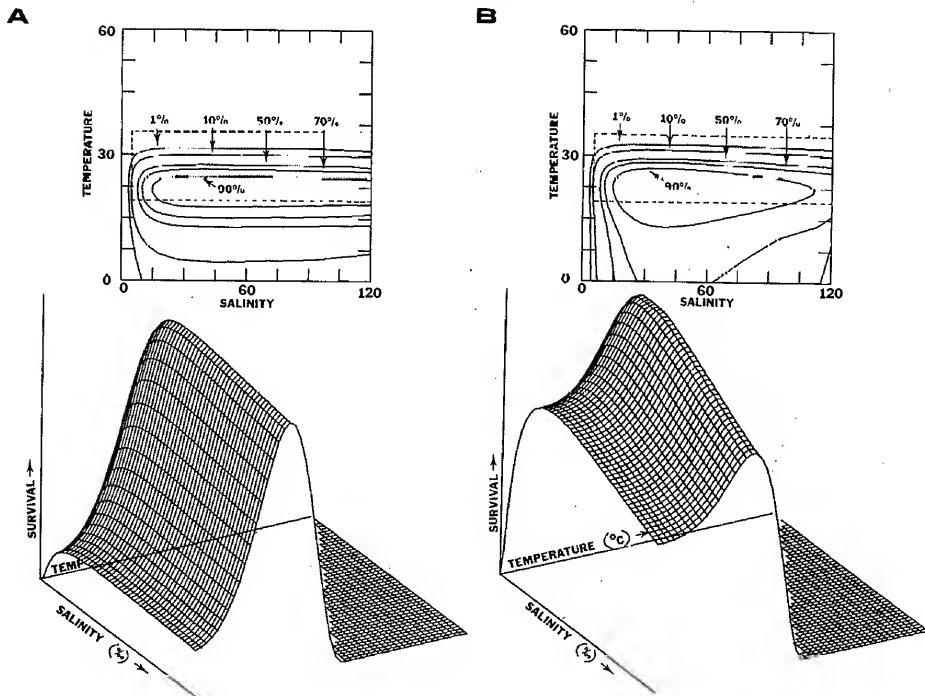


Fig. 3. Two-dimensional (upper) and three-dimensional (lower) graphical representation of response surfaces for survival of two *Artemia tunisiana* strains as a function of temperature ($^{\circ}\text{C}$) and salinity (‰): isopleths of response (1, 10, 50, 70, 90% survival); A, Larnaca; B, Barbanera.

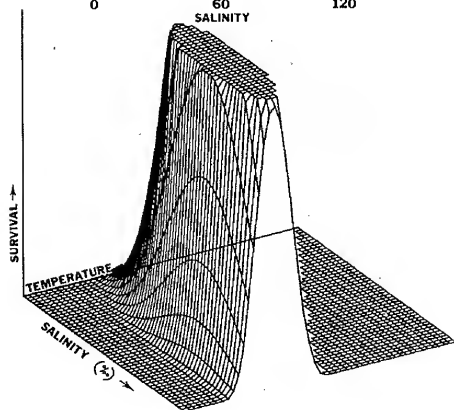
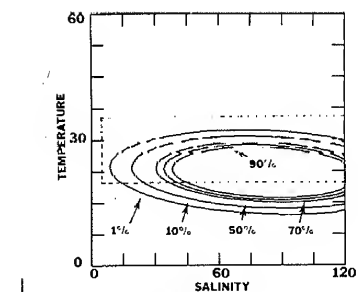
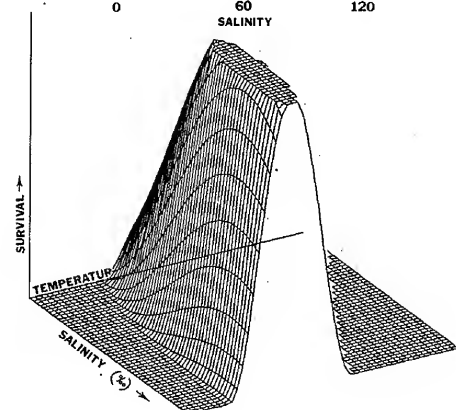
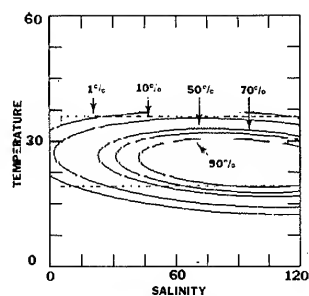
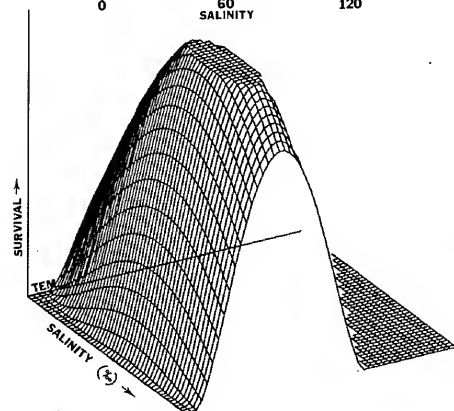
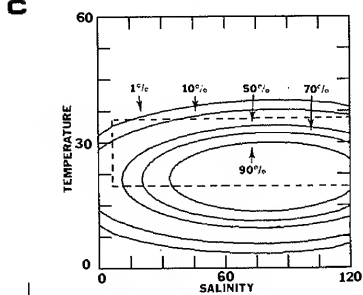
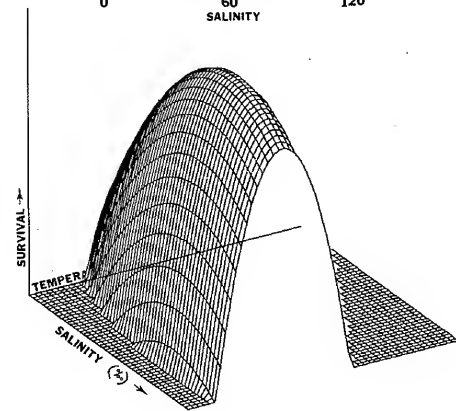
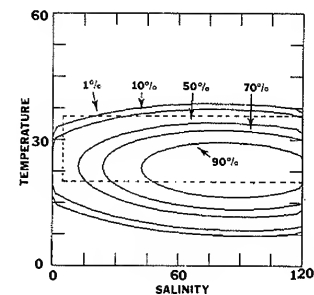
ARTEMIA PARTHENOGENETICA

Shark Bay (Fig. 4A)

From the contour plots it clearly appears that Shark Bay *Artemia* are sensitive to high temperatures and low salinities. No larvae survived at 5‰ or at 34 $^{\circ}\text{C}$. A drastic increase in mortality occurs between 26 and 30 $^{\circ}\text{C}$. The 50% survival contour does not exceed 28 $^{\circ}\text{C}$.

The calculated optimum temperature–salinity combination was 22.1 $^{\circ}\text{C}$ and 82‰. The response surface is characterized, however, by a distinct plateau. Maximal survival is attained between 40 and 120‰ and 18 and 26 $^{\circ}\text{C}$.

A significant interaction between temperature and salinity ($P = 0.025$) indicates that the Shark Bay larvae are slightly more resistant to low temperatures at higher salinities. The interaction effect is minimal in comparison with the individual effects of temperature and salinity.

A**B****C****D**

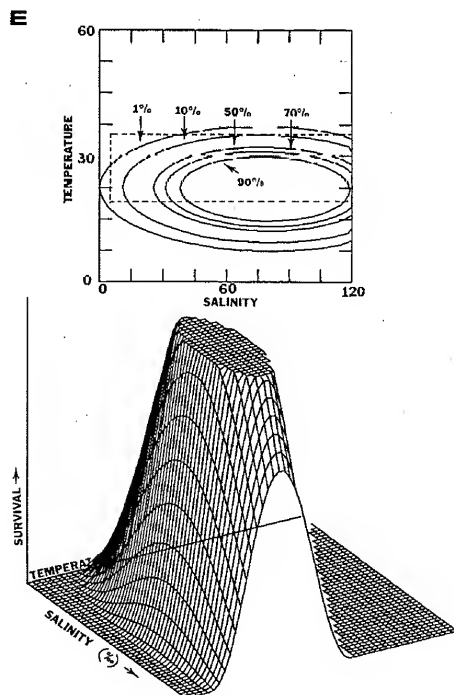


Fig. 4. Two-dimensional (upper) and three-dimensional (lower) graphical representation of response surfaces for survival of five *Artemia parthenogenetica* strains as a function of temperature ($^{\circ}\text{C}$) and salinity (‰): isopleths of response (1, 10, 50, 70, 90% survival); A, Shark Bay; B, Tuticorin; C, Lavalduc; D, Tientsin; E, Margherita di Savoia.

Tuticorin (Fig. 4B)

In comparison with the diploid Shark Bay strain the triploid parthenogenetic strain from Tuticorin seems to be more resistant to high temperatures and to a certain extent also to low salinities. Nevertheless a salinity of at least 35‰ is needed to assure good survival.

The optimum temperature for maximal survival (24.7°C) is clearly higher than the value calculated for all other strains tested. Survival as high as 90% was recorded over a wide range of temperatures ($20\text{--}29^{\circ}\text{C}$). Nevertheless, the temperature tolerance is restricted, 50% mortality occurs at $32\text{--}33^{\circ}\text{C}$. Contrary to all other strains studied, the lowest test temperature provokes significant mortalities. Royan (1980) studied the survival of Tuticorin larvae subjected for 4 days to three temperatures (24, 27, and 30°C) and seven salinities ranging from 10 to 70‰ and noted the best survival at 27°C and 30–40‰. The survival he obtained, however, was low.

The temperature–salinity interaction is significant but very limited ($P = 0.020$). The lowest temperature tested is better tolerated at the upper end of the salinity range.

Lavalduc (Fig. 4C), *Tientsin* (Fig. 4D) and *Margherita di Savoia* (Fig. 4E)

The response of these three mixed diploid-tetraploid parthenogenetic strains to temperature and salinity is very similar. Both temperature and salinity affect survival significantly, temperature being the more important. No significant interaction effect, however, was indicated for any of these strains ($P = 0.82-0.94$).

The calculated optimum combinations are: 21.8 °C, 80‰ for *Lavalduc*; 22.9 °C, 83‰ for *Tientsin*; and 22.2 °C, 79‰ for *Margherita di Savoia*. For all three strains there appears to be a well-defined range of temperatures and salinities at which >90% survival was observed ranging from 35–40 to 115–120‰ at 22 °C and from 18–29 °C at 80‰. The *Margherita di Savoia* larvae, however, show an increased mortality at 120‰.

The *Lavalduc* and *Tientsin* *Artemia* appear to be relatively tolerant to high temperatures. Survival of 50% was observed at temperatures up to 33 °C. For the *Margherita di Savoia* larvae this survival figure is already reached at 32 °C. Between 30 and 34 °C *Margherita di Savoia* larvae die rapidly. This confirms the observation of Trotta (pers. comm.) that *Margherita di Savoia* *Artemia* are completely eliminated in their biotope when water temperatures exceed 30 °C.

DISCUSSION

In all *Artemia* strains studied both temperature and salinity significantly affect survival, the effect of temperature being more pronounced. Nevertheless, no single *Artemia* strain seems to be stenothermal or stenohaline. In fact, a broad range of temperatures and salinities meet the requirements for >90% survival. With the exception of the Chaplin Lake strain, all *Artemia* strains studied have a common area of preference where mortalities are <10% (Fig. 5).

The exceptional characteristics of the Canadian *Artemia* are very likely to be attributed to the ionic composition of Chaplin Lake. Contrary to the other strains studied, all of which originate from chloride biotopes, Chaplin Lake *Artemia* live in a sulphate lake (Hammer, 1978). Apparently a culture medium with ionic composition similar to that of sea water is not optimal for this particular strain; in fact the overall survival of Chaplin Lake larvae is significantly lower than that of all other strains.

An increased mortality of nauplii originating from sulphate and carbonate lakes in a chloride medium has also been recorded by Collins (1980) and Dana (1981). In addition, Vanhaecke *et al.* (1983) reported that the survival capacity in sea water of unfed Chaplin Lake larvae is lower than that of all other strains studied. The ionic composition of Chaplin Lake, primarily a Na_2SO_4 medium, may also explain the low tolerance to extreme salinities. As compared with a NaCl medium the osmotic pressure of a Na_2SO_4 medium at the same concentration (g/l) is indeed much lower. Since the salinity level in Chaplin Lake is similar to the values reported for most other *Artemia* biotopes (Hammer, 1978; Vanhaecke, 1983), it is very likely that the osmoregulatory

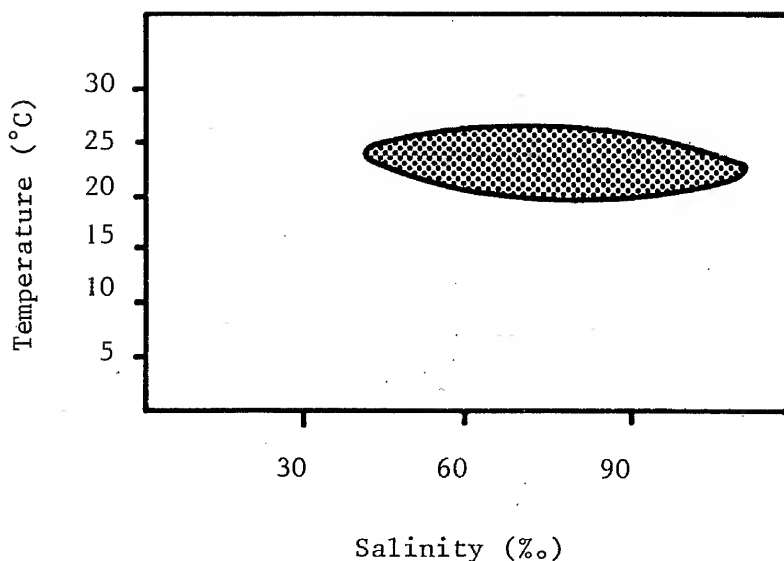


Fig. 5. Common temperature salinity area for *Artemia* strains studied in which survival exceeds 90%.

system of Chaplin Lake *Artemia* may not be able to cope with high concentrations of NaCl. At low salinities on the other hand the very low SO_4^{2-} concentration in a sea-water medium may result in an unbalanced ionic composition. Further research on the osmoregulation and ionic prerequisites of this particular strain should be carried out to verify the latter hypotheses.

Although for the other strains salinity resistance is high, a tolerance range from 3 to 300‰ as generalized for the genus *Artemia* is not valid for all *Artemia* strains. In particular at the lower end of the salinity scale distinct differences can be seen among strains. At 5‰ very high mortalities occur, confirming the findings from Von Hentig (1971) and Kristensen & Hulscher-Emeis (1972). From an analysis of variance of all survival data at 5‰, grouped per strain, it appears that Buenos Aires larvae perform significantly better at this low salinity level than all other strains tested. Since Buenos Aires, Barbanera, Larnaca, and Lavalduc *Artemia* show no increased mortality at 15‰, these strains should be selected by preference for culturing purposes in low salinity water, e.g. geothermal sources (Brisset *et al.*, 1982).

With regard to temperature, most strains appear to tolerate the lowest temperatures very well with the exception of the Tuticorin strain. At the upper end of the temperature scale most larvae die between 30 and 34 °C, which confirms the field observations of Anderson (1958), Al-Uthman (1971), Kristensen & Hulscher-Emeis (1972) and Scelzo & Voglar (1980). Substantial strain differences, however, exist with regard to high temperature resistance; for the larvae from Larnaca, Barbanera and Shark Bay, mortality starts at 30 °C and is practically 100% at 34 °C. The latter phenomenon also applies to Buenos Aires larvae. The strains from San Francisco Bay, Macau, Barotac

Nuevo and Chaplin Lake also show increased mortality at 30 °C but a number of nauplii survive at 34 °C. No significant increase in mortality was observed at 30 °C for the strains of Tuticorin, Tientsin, Lavalduc, Margherita di Savoia, and Great Salt Lake in the optimal salinity range. An analysis of variance on all survival data at 34 °C grouped per strain reveals that the survival of the Great Salt Lake strain is superior to that of all other *Artemia* strains. In practice this means that Great Salt Lake *Artemia* are best for applications in tropical and subtropical regions, where water temperatures of 30 to 35 °C are common (De los Santos *et al.*, 1980; Vos *et al.*, 1984).

There are good indications that differences in temperature resistance are correlated with genetic differentiation between and within sibling species. Both European bisexual strains (*Artemia tunisiana*, Bowen *et al.*, 1978; Abreu-Grobois & Beardmore, 1982) show the lowest tolerance to high temperatures. It is interesting to note that these strains also show the lowest temperature resistance with respect to the hatchability of their cysts (Vanhaecke, 1983). The parthenogenetic strains are more tolerant to high temperatures. The diploid Shark Bay *Artemia*, however, have a significantly lower resistance than the triploid and mixed diploid-tetraploid strains studied. This confirms reports in the literature that polyploid populations are genetically better buffered against extreme environmental conditions than diploid animals (Artom, 1931; Chapman, 1968; Metalli & Ballardin, 1972).

The difference in temperature tolerance between bisexual and parthenogenetic strains of European origin is very well related to their seasonal occurrence. Indeed Amat Domenech (1982) observed that in Spanish *Artemia* biotopes where bisexual and parthenogenetic *Artemia* co-occur, the former strain dominates in winter and spring, whereas in the warmest months of the summer parthenogenetic *Artemia* are found.

The data for the *Artemia franciscana* strains clearly illustrate differences in tolerance within the same sibling species. This is not unlikely since a marked degree of genetic differentiation may occur among strains belonging to the same sibling species (Abreu-Grobois & Beardmore, 1980, 1982). On the other hand, the organisms may have genetically adapted to their specific niche (Kinne, 1970). Since the Macau and Barotac Nuevo *Artemia* strains in fact are of San Francisco Bay origin, but are produced in saltworks with higher water temperatures (Vos *et al.*, 1984) the increased temperature resistance of Macau and Barotac Nuevo *Artemia* reveal a heritable adaptation to high water temperatures.

The data obtained are too limited to perform a detailed correlation between the temperature resistance and the temperature conditions in the biotope. In any case, water temperatures in Chaplin Lake and San Francisco Bay never exceed 30 and 33 °C, respectively (Hammer & Haynes, 1978; Carpelan, 1957), whereas in Great Salt Lake summer temperatures up to 40 °C have been recorded (Stephens & Gillespie, 1972).

ACKNOWLEDGEMENTS

this study was supported by the Belgian National Science Foundation (N.F.W.O.) through Grant F.K.F.O. 2.0012.82. We are indebted to Miss Lieve Aertsen for technical assistance. Patrick Sorgeloos is Senior Scientist with N.F.W.O.

REFERENCES

- ABREU-GROBOIS, F.A. & J.A. BEARDMORE, 1980. International study on *Artemia*. II. Genetic characterization of *Artemia* populations – an electrophoretic approach. In, *The brine shrimp Artemia. Vol. 1. Morphology, genetics, radiobiology, toxicology*, edited by G. Persoone *et al.* Universa Press, Wetteren, Belgium, pp. 133–146.
- ABREU-GROBOIS, F.A. & J.A. BEARDMORE, 1982. Genetic differentiation and speciation in the brine shrimp *Artemia*. In, *Mechanisms of speciation*, Alan Liss Inc., New York, pp. 345–376.
- ALDERDICE, D.F., 1972. Factor combinations. Responses of marine poikilotherms to environmental factors acting in concert. In, *Marine ecology, Vol. 1 (3)*, edited by O. Kinne, Wiley Interscience, London, pp. 1659–1722.
- AL-UTHMAN, H.S., 1971. Ecological observations on *Artemia salina* (L.) (Anostraca: Artemiida) in Iraq. *Bull. Biol. Res. Cent. (Baghdad)*, Vol. 5, pp. 49–65.
- AMAT DOMENECH, F., 1982. Zygogenetical and parthenogenetical *Artemia* in the Cadiz sea side salterns. Paper presented at the *International Symposium on Coastal Lagoons*, 8–14 Sept. 1982, Bordeaux (France).
- ANDERSON, G.C., 1958. Some limnological features of a shallow saline meromictic lake. *Limnol. Oceanogr.*, Vol. 3, pp. 259–270.
- ARTOM, C., 1931. L'origine e l'evoluzione della partenogenesi attraverso i differenti biotipi di una specie collettiva (*Artemia salina* L.) con speciale riferimento al biotipo diploid partenogenetico di Sète. *Mem. R. Accad. Ital.*, Vol. 2, pp. 1–57.
- BARIGOZZI, C. & M. TOSI, 1959. New data on tetraploidy of amphigonic *Artemia salina* Leach and on triploids resulting from crosses between tetraploids and diploids. *Atti Ass. genet. ital., Ric. Sci. Suppl.*, Vol. 29, pp. 129–132.
- BAYLY, I.A.E., 1972. Salinity tolerance and osmotic behavior of animals in athalassic saline and marine hypersaline waters. In, *Annual review of ecology and systematics. Vol. 3*, edited by R.F. Johnston, Annual Reviews Inc., Palo Alto, California, pp. 233–268.
- BOWEN, S.T. & G. STERLING, 1978. Esterase and malate dehydrogenase isozyme polymorphisms in 15 *Artemia* populations. *Comp. Biochem. Physiol.*, Vol. 61B, pp. 593–595.
- BOWEN, S.T., J.P. DURKIN, G. STERLING & L.S. CLARK, 1978. *Artemia* hemoglobins: genetic variation in parthenogenetic and zygogenetic populations. *Biol. Bull. (Woods Hole, Mass.)*, Vol. 155, pp. 273–287.
- BRISSET, P., D. VERSICHELE, E. BOSSUYT, L. DE RUYCK & P. SORGELOOS, 1982. High density flow-through culturing of brine shrimp, *Artemia* on inert feeds. Preliminary results with a modified culture system. *Aquac. Eng.*, Vol. 1, pp. 485–492.
- CARPELAN, L.H., 1957. Hydrobiology of the Alviso salt ponds. *Ecology*, Vol. 38, pp. 375–390.
- CHAPMAN, J., 1968. The relative adaptive values of parthenogenesis and zygogenesis. M.Sc. Thesis, San Francisco State College, California, 76 pp.
- CLARK, L.S. & S.T. BOWEN, 1976. The genetics of *Artemia*. VII. Reproductive isolation. *J. Hered.*, Vol. 67, pp. 385–388.
- CLAUS, C., F. BENJTS & P. SORGELOOS, 1977. Comparative study of different geographical strains of the brine shrimp *Artemia salina*. In, *Fundamental and applied research on the brine shrimp, Artemia salina (L.) in Belgium*. EMS Special Publication No. 2, edited by E. Jaspers & G. Persoone, Institute for Marine Scientific Research, Bredene (Belgium), pp. 91–105.
- COLLINS, N.C., 1980. Comparison of *Artemia* strains: survival and growth of nauplii as a function of ionic composition, osmosity and temperature of the medium. In, *The brine shrimp Artemia. Vol. 3. Ecology, culturing, use in aquaculture*, edited by G. Persoone *et al.*, Universa Press, Wetteren, Belgium, p. 123.

- D'AGOSTINO, A. S. & L. PROVASOLI, 1968. Effects of salinity and nutrients on mono and diaxenic cultures of two strains of *Artemia salina*. *Biol. Bull. (Woods Hole, Mass.)*, Vol. 134, pp. 1-14.
- DANA, G. L., 1981. Comparative population ecology of the brine shrimp *Artemia*. M.Sc. thesis, San Francisco State University, California, 125 pp.
- DE LOS SANTOS JR., C., P. SORGELOOS, E. LAVINA & A. BERNARDINO, 1980. Successful inoculation of *Artemia* and production of cysts in man-made salterns in the Philippines. In, *The brine shrimp Artemia. Vol. 3. Ecology, culturing, use in aquaculture*, edited by G. Persoone et al., Universa Press, Wetteren, Belgium, pp. 159-163.
- DIETRICH, G. & K. KALLE, 1963. *General oceanography*, Interscience, New York (See Kinne, 1970).
- DOBBELEIR, J., 1977. Vergelijkende studie van enkele geografische rassen van het pekelkreeftje, *Artemia salina* (L.). M.Sc. thesis, State University of Ghent, Ghent, 68 pp.
- HAMMER, U. T., 1978. The saline lakes of Saskatchewan. III. Chemical characterization. *Int. Rev. Gesamten Hydrobiol.* Vol. 63, pp. 311-335.
- HAMMER, U. T. & R. C. HAYNES, 1978. The saline lakes of Saskatchewan. II. Locale, hydrography and other physical aspects. *Int. Rev. Gesamten Hydrobiol.* Vol. 63, pp. 179-203.
- HERNANDORENA, A., 1974. Effects of salinity on nutritional requirements of *Artemia salina*. *Biol. Bull. (Woods Hole, Mass.)*, Vol. 146, pp. 238-248.
- IVLEVA, I. V., 1969. Branchiopoda. In, *Mass culture of invertebrates. Biology and methods*. Acad. Sci. of the USSR, All-Union Hydrobiological Society, Izdatel'stvo "Nauka", Moskva. Translated into English by "Israel Program for Scientific Translations", Jerusalem (Israel) 1973, pp. 62-95.
- KINNE, O., 1970. (Editor), *Marine ecology, Vol. 1. Environmental factors*. Wiley-Interscience, J. Wiley & Sons, London, 681 pp.
- KRISTENSEN, I. & T. M. HULSCHER-EMEIS, 1972. Factors influencing *Artemia* populations in Antillean salines. *Stud. Fauna Curacao*, Vol. 39, pp. 87-111.
- LINDSEY, J. K. & A. M. SANDNES, 1972. Program for the analysis of non-linear response surfaces (Version III). *Fish. Res. Board. Can. Tech. Rep.*, No. 311, 145 pp.
- METALLI, P. & E. BALLARDIN, 1972. Radiobiology of *Artemia*: radiation effects and ploidy. *Curr. Top. Radiat. Res. Q.*, Vol. 7, pp. 181-240.
- PERSOONE, G. & P. SORGELOOS, 1980. General aspects of the ecology and biogeography of *Artemia*. In, *The brine shrimp Artemia. Vol. 3. Ecology, culturing, use in aquaculture*, edited by G. Persoone et al., Universa Press, Wetteren, Belgium, pp. 3-24.
- PERSOONE, G., P. SORGELOOS, O. ROELS & E. JASPERS, 1980. (Editors), Editorial note on the taxonomy of *Artemia*. In, *The brine shrimp Artemia. Vol. 1. Morphology, genetics, radiobiology, toxicology*, Universa Press, Wetteren, Belgium, pp. xvii-xviii.
- PROVASOLI, L. & A. S. D'AGOSTINO, 1969. Development of artificial media for *Artemia salina*. *Biol. Bull. (Woods Hole, Mass.)*, Vol. 136, pp. 434-453.
- ROYAN, J. P., 1980. Laboratory and field studies on an Indian strain of the brine shrimp *Artemia*. In, *The brine shrimp Artemia. Vol. 3. Ecology, culturing, use in aquaculture*, edited by G. Persoone et al., Universa Press, Wetteren, Belgium, pp. 223-230.
- SCELZO, H. A. & J. F. VOGLAR, 1980. Ecological study of the *Artemia* populations in Boca Chica salt lake, Margherita Island, Venezuela. In, *The brine shrimp Artemia. Vol. 3. Ecology, culturing, use in aquaculture*, edited by G. Persoone et al., Universa Press, Wetteren, Belgium, pp. 115-126.
- SIDDALL, S. E., 1979. Temporal changes in the salinity and temperature requirement of tropical mussel larvae. In, *Proc. World Maricult. Soc.*, edited by J. W. Avault Jr., Vol. 9, pp. 549-566.
- SORGELOOS, P., 1979. Het gebruik van het pekelkreeftje *Artemia* spec. in de aquakultuur. Special doctorate thesis, State University of Ghent, Ghent, 320 pp.
- SORGELOOS, P., 1980. The use of the brine shrimp *Artemia* in aquaculture. In, *The brine shrimp Artemia. Vol. 3. Ecology, culturing, use in aquaculture*, edited by G. Persoone et al., Universa Press, Wetteren, Belgium, pp. 25-46.
- SORGELOOS, P., M. BAEZA-MESA, F. BENIJTS & G. PERSOONE, 1976. Current research on the culturing of the brine shrimp *Artemia salina* L. at the State University of Ghent, Belgium. In, *Proc. 10th Europ. Symp. Mar. Biol., Vol. 1. Research in mariculture at laboratory and pilot scale*, edited by G. Persoone & E. Jaspers, Universa Press, Wetteren, Belgium, pp. 472-495.
- SORGELOOS, P., E. BOSSUYT, E. BRUGGEMAN, A. COOREMAN, J. DOBBELEIR, P. VANHAECKE & D. VERSICHELE, 1979. Progress report on the research activities of the *Artemia* Reference Center. *ICES C.M./F.* 54, pp. 206-214.

- STEPHENS, D. W. & D. M. GILLESPIE, 1972. Community structure and ecosystem analysis of the Great Salt Lake. In, *The Great Salt Lake and Utah's water resources*, edited by J. P. Riley, Utah Water Res. Lab., Utah State University, pp. 66-72.
- VANHAECHE, P., 1983. Vergelijkende studie van diverse geografische rassen van het pekelkreeftje *Artemia* ter verbetering van zijn gebruik in de aquakultuur. Ph. D. thesis, State University of Ghent, Ghent, 420 pp.
- VANHAECHE, P., P. LAVENS & P. SORGELOOS, 1983. International study on *Artemia*. XVII. Energy consumption in cysts and early larval stages of various geographical strains of *Artemia*. *Ann. Soc. R. Zool. Belg.*, Vol. 113, pp. 155-164.
- VON HENTIG, R., 1971. Einfluss von Salzgehalt und Temperatur auf Entwicklung, Wachstum, Fortpflanzung und Energiebilanz von *Artemia salina*. *Mar. Biol.*, Vol. 9, pp. 145-182.
- VOS, J., PH. LEGER, P. VANHAECHE & P. SORGELOOS, 1984. Quality evaluation of brine shrimp *Artemia* produced in Asian salt ponds. *Hydrobiologia*, in press.
- ZAR, J., 1974. *Biostatistical analysis*. Prentice-Hall Inc., Englewood Cliffs, New Jersey, 620 pp.

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